The Second International Conference on Food and Agriculture
ISBN : 978-602-14917-9-9

PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY FROM FRUIT OF KULIM (*SCORODOCARPUS BORNEENSIS* BECC.)

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Abstract. This research was carried out to determine the phytochemical and antioxidant activity from the fruit of Kulim (*Scorodocarpus borneensis* Becc.). The partition of extract was prepared by fractionation using gradient elution with hexane, ethyl acetate, ethanol, methanol, and 70% methanol respectively and stirred for 24 hours. The crude fraction then concentrated using rotary evaporator at 40□. Qualitative determination of fruit fractions by phytochemical screening were tested for the presence of flavonoids, alkaloids, tannins, phenols and terpenoids, while quantitative determination of total phenol and antioxidant activity by DPPH free radical scavenging activity was carried out using colorimetric methods. The yields of crude fraction were obtained 0,47% (hexane), 0,09% (ethyl acetate), 0,30% (ethanol), 1,23% (methanol) and 1,89% (70% methanol). Phytochemical screening of S. borneesis fruit crude fractions revealed the presence of all tested variable on ethanol, methanol and 70% methanol solvent. The highest total phenolic content and antioxidant activity of Kulim fruits was obtained from fractionation with methanol solvent, that is 927,36 mgGAE/g and 14,54ppm respectively.

Keywords : *S.borneensis*, fractionation, phytochemical, antioxidant.

Introduction

The use of plant compounds for pharmaceutical purposes has gradually increased in the world. Plants are very good sources of medicinal compounds that have continued to play a dominant role in the maintenance of human health since ancient times (Moriita *et.al.,* 2011). There is a wide diversity of compounds, especially secondary metabolites, found and isolated from plants and studies have shown that these compounds have anticancer, antibacterial, analgesic, anti-inflammatory, antitumor, antiviral and many other activities to a greater or lesser extent (Cai *et al*., 2004; Miliauskas *et al*., 2004). Distinguished examples of these phytochemical compounds include flavonoids, phenols and phenolic glycosides, saponins and cyanogenic glycosides, stilbenes, tannins, nitrogen compounds (alkaloids, amines, betalains), terpenoids and some other endogenous metabolites (Cai *et al.*, 2004; Abdulwahab *et al.*, 2011). Those phytochemical compounds can be obtained from the plant by extraction with solvent.

Scorodocarpus borneensis was one of olacaceae family plant that distributed and grew only at Sumatera and Borneo Island (Sleurman, 1982). Olacaceae is known as a producer of tannin, glycoside cyanogenetic, polyacetylenic fatty acids, flavonoids and a series of polysulfide compounds (Wiart *et al.*, 2001). This plant is a tall tree which has been named by natives as “wood garlic” due to its strong garlic-like smell. This garlic smell is present in leaves, flowers and fruit. The fallen fruit has a hard outer nutshell, and is similar in shape and size to a walnut (Kubota *et al*., 1994) The natives of Sabah

ISBN : 978-602-14917-9-9

in Malaysia occasionally use the sliced or dry-powdered pulp of the fruit as a seasoning just like garlic for cooking some types of fish (Burkill, 1935).

According to Lim *et al.* (1999), the fruit of the wood garlic contains sulfur compounds, such as 2,4,5-trithiahexane and 2,4,5,7-tertrathioctane 4,4-dioxide. These chemical compounds showed inhibitory effect on the metabolism of arachidonic acid and suppressed clumping of platelets in the blood cells of rabbits. This fruit extract can be used to control some types of fungi in wood building, such as *Candida albicans*, *Saccharomyces cerevisiae*, *Mucor racemosus* and *Aspegillus niger*. The bioactive compounds are 2,4,5,7-tetrathiaoctane 4,4-dioxide; 5-thio-2,4,6-trihiaheptane 2,2-dioxide; 0-ethyl-S methyl-thiomethyl thiosulfite (Verma *et al.*, 2008).

The research about antimicrobial and anticancer of *S.borneensis* fruit were conducted before but there is no report about the phytochemical and antioxidant activity of *S.borneensis* fruit extract. The objective of this research was carry out phytochemical screening and determine the total phenol and antioxidant activities by DPPH free radical scavenging method of *S.borneensis* fruit extract that extracted continuously with different solvents. The goal of this research is to find out the best solvent to extract the fruit that possess the highest antioxidant activity and phenolic content.

2. Materials and methods

2.1. Plant materials and preparation

The fruits of *S.borneensis* were collected from of the Kulim tree were collected from local forest in Sanggau Regency, Kalimantan Barat Province, Indonesia (0°23'16.7"N and 110°43'24.8"E)**.** The materials were air dried in room temperature. The air dried fruits then finely powdered with grinder to 80-mesh size. The powder were stored in plastic jar at room temperature.

2.2. Chemical and reagents

All chemicals used were analytical grade. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), gallic acid, natrium carbonate (Na_2CO_3), folin-ciocalteu reagent, hydrochloric acid (HCl), sulfuric acid (H_2SO_4), chloroform, $FeCl_3$, aquades, methanol, ethanol, ethyl acetate, hexane, formaldehyde and natrium hydroxide (NaOH) were obtained from Sigma (Sigma-Aldrich, Germany) .

2.3. Fractionation

50 grams of *S.borneensis* fruit powder The partition of extract was prepared by fractionation using gradient elution with hexane, ethyl acetate, ethanol, methanol, and 70% methanol respectively and stirred for 24 hours. The crude fractions was filtered through Whatman No.1 filter paper then concentrated using rotary evaporator at 40□. This fractions were stored in freezer until further analysis.

2.4. Pytochemical screening

The crude fractions of *S. borneensis* fruit were tested for the presence of flavonoids, alkaloids, tannins, phenols and terpenoids based on the method described by Prabhavathi *et al.* (2016). The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals.

2.4.1. Flavonoids

2 ml of crude fraction was treated with 1ml of 10% aqueous NaOH. The appearance of intense yellow colour which turned colourless on addition of HCl, indicated the presence of flavonoids.

2.4.2. Alkaloids

Each 1 ml of crude fractions was added with 1 ml of marquis reagent (1ml conc. H_2SO_4 and 1 drop of formaldehyde) followed by 2 ml conc. H_2SO_4 and few drops of formaldehyde. The formation of orange or dark violet colour indicated the presence of alkaloids.

2.4.3. Tannins

ISBN : 978-602-14917-9-9

Each 2 ml of crude fractions was added with 10 % methanolic $FeCl_3$. The appearance of dark or brownish blue indicated the presence of tannis.

2.4.4. Phenols

Each 2 ml crude fraction was added with 5% aqueous $FeCl_3$. The formation of blue colour indicated the presence of phenols.

2.4.5. Terpenoids

Each 1 ml crude fraction was added 0.5 ml of chloroform followed by few drop of conc. H_2SO_4. The formation of brownish red indicated the presence of terpenoids on extract.

2.5. Total Phenolic Content

Total phenolic content of crude fraction was evaluated by Farhan *et al.* (2012) with slight modification. The crude fractions were dissolved with acidified methanol (1µg/ml). An aliquot of 0.2 ml crude fractions was added with 1 ml folin ciocalteu (1:10v/v) followed by 3 ml Na_2CO_3 2%. The solution were homogenized with vortex and incubated in dark for 30 minutes. The absorbance of the mixture was measured at 765 nm. Standard calibration curve for gallic acid in the range of 0–140 mg/ml was prepared in the same procedure. The concentration of the total phenolics was calculated as mg of gallic acid equivalent (GAE) by using an equation obtained from gallic acid calibration curve.

2.6. DPPH Radical Scavenging Activity

DPPH radical scavenging activity was evaluated by method described by Yen and Chen (1995) with slight modification. 4ml extract was added with 2 ml of 0.2 mM DPPH methanolic solution and incubated for 30 minutes at room temperature in dark. The absorbance was detected at 517 nm using a UV-Vis spectrophotometer. The percent inhibition of the DPPH activity was calculated as:

$$\text{DPPH inhibition (\%)} = [(Ac - As)/ Ac] \times 100$$

Where Ac = absorbance of the control (blank) and As = absorbance of the extract. The antioxidant activity represented as the 50% inhibition (IC_{50}) value.

3. Results

Plants contain secondary metabolites such as polyphenols that has benefits for human body, that is anticancer, antibacterial, anti-inflammatory, antiviral and many other activities. Phenolic compounds are a class of antioxidant agents that can inhibit and scavenge free radicals due to their redox properties (Jimoh *et al*., 2011). Different solvents possess different extraction abilities, which are dependent on the polarity of the extraction medium and the type of solvents used (Alothman *et al.,* 2009). The present study revealed that the distribution of polyphenols different between all solvents.

The percentage yield of the crude fractions in decreasing order is as follows 1.89% (70% methanol), 1.23% (methanol), 0.47% (hexane), 0.30% (ethanol) and 0.09% (ethyl acetate). Total phenolic of all fractions were ranged from 52.01 to 927.36 mg/GAE and IC_{50} DPPH antioxidant activity were ranged from 14.54 to 888.60 ppm.

Table 1. Total Phenolic Content of *S.borneensis* fruit fractions with different solvent (as mg Gallic Acid Equivalent)

Solvent	TPC (mgGAE/g)
Hexane	52.01 ± 23.21
Ethyl Acetate	277.01 ± 5.74
Ethanol	862.78 ± 21.68
Methanol	927.36 ± 46.26
70% Methanol	132.57 ± 13.75

ISBN : 978-602-14917-9-9

Table 2. Radical Scavenging Activity of *S.borneensis* fruit fractions by DPPH method with different solvent (IC_{50})

Solvent	IC_{50}(ppm)
Hexane	888.60 ± 64.43
Ethyl Acetate	20.21 ± 0.48
Ethanol	14.88 ± 1.06
Methanol	14.54 ± 0.48
70% Methanol	170.35 ± 8.48

4. Discussion

4.1. Phytochemical screening

The phytochemical screening of *S.borneensis* fruit fractions were revealed the presence of secondary metabolite such as alkaloids, flavonoids, tannis, phenols and terpenoids as shown in Table 3. Terpenoids were presence on ethanol, methanol and 70% methanol fractions but not on ethyl acetate and hexane fraction. The phytochemical compounds detected are known to have medicinal effect. For example many alkaloids derived from medicinal plants show biological activities like, anti-inflammatory (Augusto *et al.*, 2011) antimalarial (Dua *et al.*, 2013) and antimicrobial (Benbott *et al.*, 2012). Tannins, according to research, are known to have antibacterial (Hisanori *et al.*, 2001), antitumor and antiviral activities (Kumari and Jain, 2012)

Tabel 3. Phytochemical Screening of *S.borneensis* Fruit Fractions with Different Solvent

Solvent	Flavonoids	Alkaloids	Tannins	Phenols	Terpenoids
Hexane	+	+	+	+	-
Ethyl Acetate	+	+	+	+	-
Ethanol	+	+	+	+	+
Methanol	+	+	+	+	+
70% Methanol	+	+	+	+	+

Note : + = present ; - = absent

Flavonoid and phenols acts as good scavengers of lethal radicals thus preventing oxidative cell damage. They are also known to have various pharmacological and biological activities such as anti-inflammatory, immunomodulatory and anti-allergic properties which reduce the risk of cardiovascular mortality (Olajuyigbe and Afolayan, 2011).

4.2 Total Phenolic Content

Total phenolic content of crude fractions are presented in Table 1. The phenolic content of crude fractions in decreasing order is as follows methanol >ethanol >ethyl acetate >70% methanol>hexane. According Sousa *et al*. (2007), the phenolic compounds are distributed in the following categories: Simple phenolics, phenolic acids (benzoic and cinnamic acid derivatives), coumarins, flavonoids, hydrolysable and condensed tannins, stilbenes, lignans, and lignins. They have the ability to inhibit lipid peroxidation and lipoxygenase *in vitro*. Consumption of flavonoid containing fruits and vegetables has been linked to protection against cancer and heart disease (Hertog *et al*., 1992; Atoui *et al*, 2005). The higher total phenolic content, the higher the antioxidant activity (Huang *et al.*, 2005). According to Ramma *et al*. (2002), different part of plant and physiological time of those plant were affect the phenolic content.

4.3. DPPH Radical Scavenging Activity

The antioxidant ability and radical scavenging properties of plants are associated with its medicinal values (Cai *et al.*, 2004). The DPPH is a stable free radical which has been widely used to evaluate the antioxidant activity of extracts and pure substances. The effect of antioxidants on the DPPH is due to Hydrogen

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donor capacity of these substances which are usually phenolic compounds (Souza, 2006). The radical scavenging activity of *S. borneensis* fruit fractions by DPPH method are presented in Table 2. DPPH and the free radical scavenging activity of the fractions were found to be in the order of methanol > ethanol >ethyl acetate > 70% methanol > hexane. This order is similar to the phenolic contents of the fractions that showed the antioxidant activity of the extract is in accordance with the amount of phenolics present in that extract (Abdille *et al.*, 2005).

Several reports have finally shown close relationship between total phenolic contents and antioxidative activity of the fruits, plants and vegetables (Deighton *et al.*, 2000; Abdille *et al.*, 2005; Vinson *et al.*, 1998). The high antiradical property of extract may be due to the presence of the phenolic compound (Majouli *et al.*, 2017). The quality of the obtained extracts was affected mainly by the solvent used for extraction. Methanol is the best solvent for preparing herbal infusions, yielding the strongest antioxidant activity in the extract. The more the polar capacity of the extract, the greater is its antioxidant activity (Mon *et al.*, 2011).

5. Conclusion

Fruit of *S.borneensis* Becc. represent rich of phytochemical such as polyfenol that exhibited high antioxidant activity which is potential to be explored for medical purpose.

6. Acknoledgement

The authors would like to thank to The Ministry of Research, Technology and Higher Education (Kemenristekdikti) for the research grant.

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